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Local medium effects in the photochemical behavior of substituted stilbenes immobilized on quartz surfaces

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Abstract

Several substituted *trans*-stilbene derivatives have been prepared and immobilized onto a quartz surface. A number of immobilization methods has been tried including the silanization technique, cross-linking with cyanuric chloride, surface activation with cyanogen bromide and surface smoothing with coating proteins. Direct cross-linking failed, but other immobilization techniques were successful. Studies of solvent polar effects on the fluorescence spectrum of the immobilized stilbenes indicate that the maximum wavelength of the fluorescence emission is not very sensitive to solvent polarity. The apparent local polarity of the medium in the vicinity of the stilbene label was estimated, and E_T^{30} value was found to be close to 50 kcal mol^{-1}. The *trans–cis* photoisomerization kinetics of the stilbene derivatives in the immobilized and free state in a medium of different viscosity was monitored with the fluorescence technique at constant-illumination conditions. The apparent photoisomerization rate constant of the process was calculated using steady-state approximations. It was found to be 3–4 times less for the immobilized label than in a free state which indicates that the surface and protein itself sterically hinder the rotation of the stilbene fragment around the olefinic double bond in the excited state. Investigation of the microviscosity effect on the photoisomerization of the immobilized and free stilbene label was carried out by changing the relative concentration (% v/v) of glycerin in a glycerin–water mixture used as a solvent. With the appropriate calibration, the microviscosity in the vicinity of the stilbene label was estimated.

Keywords: Stilbene; *trans–cis* photoisomerization; Immobilization; Surface modification; Silanization; Cyanogen bromide; Micropolarity; Microviscosity; Fluorescence-photochrome method

1. Introduction

Many processes concerning daily life occur at surfaces, including life itself. Even if we limit our attention to solid surfaces, the importance of the processes is hardly reduced. The properties and reactivities of molecules attached to slid surfaces have been the subject of numerous recent investigations [1–6]. There has been particular interest in the photochemical and photophysical behavior of such molecules and their interaction with other molecules attached to the same surface due to widespread potential applications as the photo-sensitive devices [7–10].

Stilbene and a variety of diarylethylenes have been the subject of intense study insofar as their solution photochemistry and photophysics are concerned [11–25]. These molecules offer several advantages as potential probes in different organized media. The first advantage is their well-characterized photochemical reactivity which in a dilute solution consists mainly of fluorescence and *trans–cis* isomerization for *trans*-stilbenes having weak donor–acceptor substituents at their aromatic rings. The *trans–cis* isomerization is the main quenching funnel of fluorescence. It proceeds from the lowest exited singlet state $^1t^*$ through the twisted singlet intermediate $^1p^*$:

$$^1t^* \rightarrow {}^1p^* \rightarrow {}^1p \rightarrow (1-\beta)^1c + \beta^1t$$

or, alternatively, by the intersystem crossing pathway via the biradical twisted triplet state $^3p^*$:

$$^1t^* \rightarrow {}^3t^* \rightarrow {}^3p^* \rightarrow {}^1p \rightarrow (1-\alpha)^1c + \alpha^1t$$

Here, $^3t^*$ and $^3p^*$ are the *trans* and twisted configurations (perpendicular with respect to the C=C double bond),

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respectively, of the lowest triplet, ^{1}p is the twisted ground state, $(1-\alpha)$ is the fraction of triplet decay into the *cis* form and $(1-\beta)$ is the fraction of perpendicular singlet configuration decaying into the *cis* form.

A second attractive aspect of the stilbene chromophores is their potential ground- and excited-state reactivity with a variety of reagents. Here one anticipates that reactivity can be modified strongly by the precise microenvironment experienced by the chromophore in different assemblies. The photochemical behavior of stilbenes may also be varied internally by the introduction of substituents which effect the charge distribution of the molecule. Previously we reported our studies on photochemical and photophysical behavior of several stilbenes having the different donor–acceptor substituents at the 4- and 4′ positions of the aromatic rings in their aqueous solutions and being incorporated into the cells membranes [26,27]. In the present work, we report on their behavior at the quartz-contaminated surface.

While spin, luminescent and Mössbauer labels and probes have been proved to be very versatile in investigation of local molecular dynamics in biological systems [28–30], those methods have serious limitations in their application to investigation of processes occurring at surfaces. Nitroxide spin-labeling exhibits poor sensitivity and requires an expensive ESR experimental set-up. Fluorescence-polarization technique is also expensive and complicated because:

1. it requires the high-grade polarizing optics,
2. it loses the sensitivity at polarization of incident and emitted light,
3. it is applicable only to dynamic processes when their characteristic times commensurate the lifetime of the excited singlet state in the nanosecond range.

Finally, theoretical considerations and experimental data indicate that the processes at surfaces can be studied with the fluorescence-photochrome technique for investigation of local medium effects and phase transitions in biological and model membranes [26,28]. The method is based on monitoring the *trans–cis* photoisomerization kinetics of stilbene derivative incorporated into object of interest. The viscosity dependence of this behavior suggests that stilbene molecules can be useful indicators of local microviscosity in various condensed media [29]. It was shown the apparent rate constant of the *trans–cis* photoisomerization in viscous media, including biological membranes as well, is controlled by the medium relaxation rate. Hence, it was possible to estimate the local microviscosity of the media and the rotational frequency of the stilbene fragment in the exited singlet state [26]. In the present work, the fluorescence-photochrome method is applied for the investigation of the solid surface which is quartz-contaminated and modified with the stilbene-derivatives labels. A number of methods of surface modification have been tried including the silanization techniques, surface activation with cyanogen bromide (BrCN), cross-linking with cyanuric chloride and surface coating with proteins (lysozyme and bovine serum albumin, BSA) as smoothing intermediates. The apparent photoisomerization rate constants of the stilbene label in its immobilized and free states were measured in a medium of different viscosity. The microviscosity and micropolarity in the vicinity of the immobilized label were estimated.

2. Experimental

2.1. Materials

The specific surface of plain quartz-contaminated plates (30 mm × 8 mm, thickness of 0.96–1.06 mm, Corning Glass Works, Scientific Glassware Department) was used as a carrier.

The following commercial reagents and solvents were used: hydrofluoric acid (38–40%, Merck), sulfuric acid (96–98%, Frutarom), hydrochloric acid (32%, Frutarom), potassium dichromate ($K_2Cr_2O_7$, Aldrich), hydrogen peroxide (30% w/w solution, Frutarom), toluene (Frutarom), cyanuric chloride (Aldrich), BSA (bovine serum albumin, Sigma), γ-aminopropyltrimethoxysilane (Aldrich), acetone (Frutarom), acetonitrile (Frutarom), *N,N*-diisopropylethylamine (DIPEA) (Aldrich), chloroform (Frutarome), triethylamine (Aldrich), lysozyme (Sigma), triethyl phosphite (Aldrich), 4-methylbenzyl chloride (Aldrich), sodium hydride (60% dispersion in mineral oil, Aldrich), dimethylformamide (Frutarom), 4-tolualdehyde (Aldrich), dichloromethane (Frutarom), sodium sulfate (Frutarom), ammonium hydroxide (28%, Frutarom), *N*-bromosuccinimide (Merck), carbon tetrachloride (Frutarom), 1,1′-azobis (cyclohexanecarbonitrile) (Aldrich).

Highly fluorescent *trans*-4-dimethylamino-4′-aminostilbene was synthesized previously [26]. Cyanuric chloride was recrystallized from chloroform before use. Double-distilled water was used for washing of the silica plates.

The synthesis of *trans*-4,4′-dimethylstilbene was carried out by a modified Wittig–Horner procedure which is outlined as follows [31]. A mixture of 15 ml of 4-methylbenzyl chloride and 23.1 ml of triethyl phosphite was refluxed gently in a 100 ml round-bottomed flask fitted with an air condenser. Elimination of ethyl chloride started at about 130°C and over about 90 min the temperature of the liquid rose to 190–192°C. It was kept below 200°C to prevent the destruction of the phosphonate formed during the reaction. The crude phosphonate ester was then cooled to room temperature and poured into a 500 ml three-necked flask fitted with a thermometer, a condenser with a drying tube, a dropping funnel, and a mechanical stirrer and containing 5.5 g of sodium hydride (60% dispersion in mineral oil) and 120 ml of dry dimethylformamide (DMF). A solution of 14.1 ml of 4-tolualdehyde in 90 ml of dry DMF was added dropwise with stirring and cooling in ice at such a rate that the reaction temperature was maintained between 30°C and 40°C and a clear solution resulted. The stirring and cooling

was continued for 30 min. After standing at room temperature for an additional 30 min, the solution was poured on crushed ice to precipitate the product with plenty of water and dried. The dried crude product was dissolved in 20 ml of dichloromethane, and the solution was passed through a 35 mm × 5 mm silica gel 60 (Merck, 230–400 mesh ASTM) chromatographic column which had been washed with dichloromethane. Elution of the product *trans*-4,4′-dimethylstilbene was controlled with TLC (R_F 0.92, fluorescent spot irradiated at 366 nm with a UV lamp). After evaporation of the solvent, the purified colorless product was dried and recrystallized from ethanol yielding 9.1 g of white lustrous crystals with a mp at 180°C. The product sample was submitted for ^{1}H NMR analysis to confirm the chemical purity and structural identity. ^{1}H NMR spectrum was taken at 298 K on a 500.1 MHz Bruker Fourier transform spectrometer equipped with a DMX Avance system and Bruker UXNMR program with Me_4Si as the internal standard in 10% w/v solutions in $CDCl_3$ or DMSO-d_6. ^{1}H NMR spectrum: δ 2.35 (s, 6H, CH_3); CH=CH AA′ pattern: δ 7.04 (s, vinyl, 2H); 4,4′-CH_3-Ar AA′BB′ pattern: δ 7.14, 7.18 (d ,4H) δ 7.38, 7.42 (d, 4H).

Radical bromination of the prepared *trans*-4,4′-dimethylstilbene with *N*-bromosuccinimide in dry carbon tetrachloride in the presence of 1,1′-azobis(cyclohexanecarbonitrile) as a radical initiator affords the desired *trans*-4,4′-bis-brommethylstilbene [32]. We applied some modifications to the original procedure, i.e., use of carbon tetrachloride instead of chloroform as a reaction solvent. ^{1}H NMR spectrum of the product: δ 4.51 (s, 4H, CH_2Br); CH=CH AA′ pattern: δ 7.10 (s, vinyl, 2H); 4,4′-$BrCH_2$-Ar AA′BB′ pattern: δ 7.37, 7.41 (d, 4H), δ 7.48, 7.52 (d, 4H).

2.2. Methods

2.2.1. Silanization techniques

The quartz plates were treated with 40% HF for 5 min and then with chromic acid (H_2CrO_4), prepared from diluted sulfuric acid (20% H_2SO_4) and potassium dichromate ($K_2Cr_2O_7$), for 2 h in order to activate the surface [33]. After washing with double-distilled water, the plates were treated with a mixture of concentrated 6 M HCl and aqueous 30% H_2O_2 (4 : 1 v/v) for 2 h and then with 6 M HCl for an additional 22 h to increase the density of OH groups on the surface. After such activation, the plates were rinsed repeatedly with a copious amount of double-distilled water. Silanization of the surface was carried out by treatment of the silica plates with 10% solution of γ-aminopropyl-trimethoxysilane in toluene at 110°C overnight. The plates were rinsed thoroughly with toluene and acetone and dried in the vacuum oven overnight.

The following cross-linking with 0.01 M solution of cyanuric chloride in acetonitrile was carried out at 0–5°C for 3 h in the presence of a strong hindered non-nucleophilic base *N,N*-diisopropyl-ethylamine (DIPEA). Then the plates were washed with acetonitrile and acetone. The stilbene

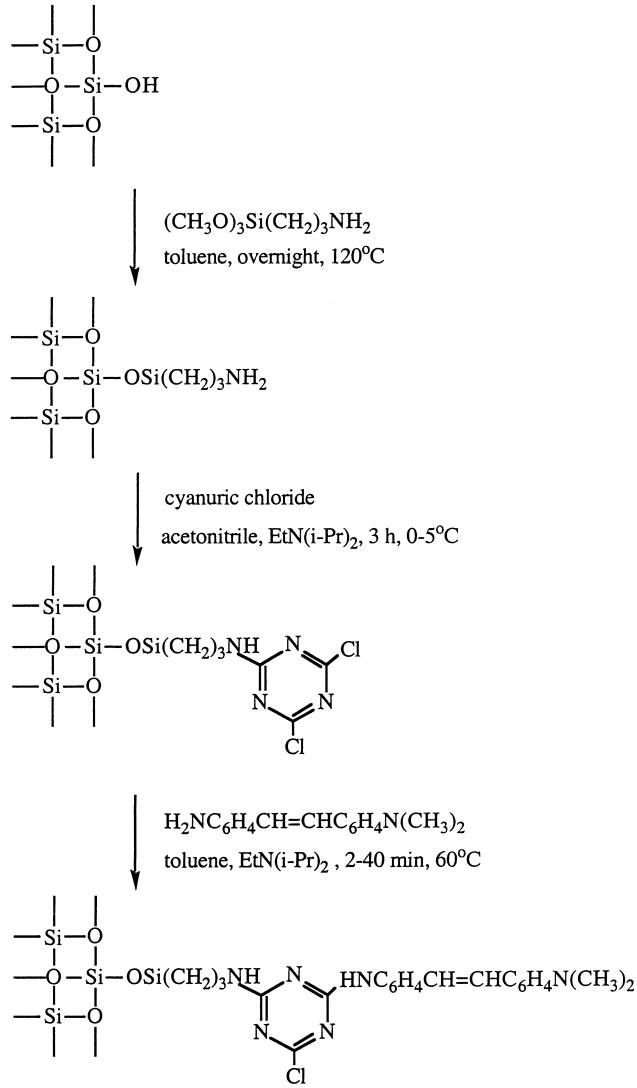

Fig. 1. Cross-linking of *trans*-4-dimethylamino-4′-aminostilbene with cyanuric chloride by the direct silanization technique.

label (*trans*-4-dimethylamino-4′-aminostilbene) was linked to the modified surface in its 5 × 10^{-4} M acetonitrile solution at 40°C for 2, 10, 20, 30 and 60 min in the presence of DIPEA, and then the plates were rinsed with acetonitrile for 48 h in order to remove all unreacted organic material. The schematic representation of the whole procedure is shown in Fig. 1.

Another method for the stilbene immobilization through coating the surface with BSA protein is shown in Fig. 2. Activation of the silica surface and subsequent silanizaition were carried out as mentioned above. The modified plates were treated with 10^{-5} M cyanuric chloride solution in acetonitrile at 3°C. To immobilize the protein, the plates were treated with the 10^{-5} M BSA solution in borate buffer (pH 9.0) for 2.5 h. The modified plates were washed with plenty of double-distilled water. Crosslinking of the cyanuric chloride to the BSA protein was carried out in a mixture of acetone and borate buffer (1 : 9 v/v) at 0–5°C for 1 h. The

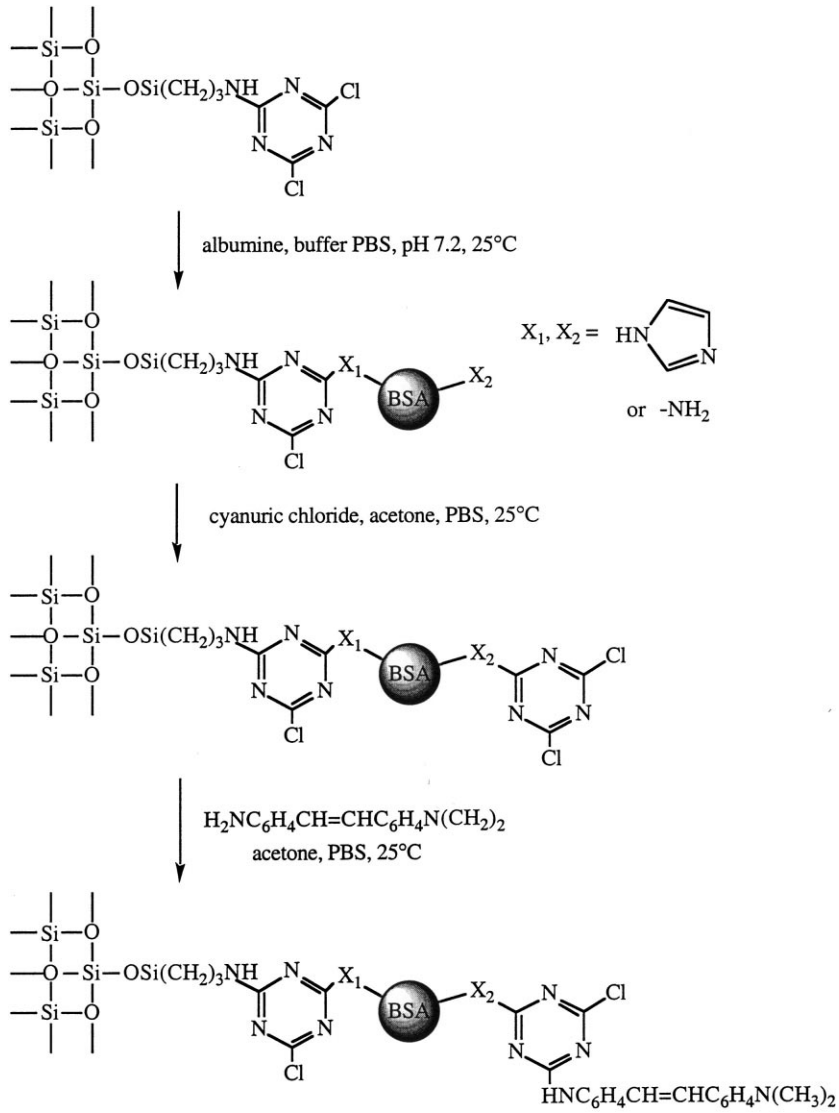

Fig. 2. Immobilization of *trans*-4-dimethylamino-4′-aminostilbene cross-linked with cyanuric chloride to the surface coated with bovine serum albumin.

concentration of cyanuric chloride in the mixture was 10^{-5} M. After that, the plates were washed thoroughly with double-distilled water. Finally, the modified plates were treated with the stilbene label (2×19^{-6} M solution in a mixture of 1 : 9 v/v acetone and borate buffer) for 30 min at ambient temperature. Again, the silica plates were rinsed with double-distilled water for 2 days in order to remove all the impurities from the surface.

2.2.2. *Surface activation with cyanogen bromide*

The procedure was carried out at room temperature. Quartz was activated with cyanogen bromide according to the modified Kohn and Wilchek method [34,35]. Previously, the quartz-contaminated plates were treated with a 1 M HCl solution for 1 h and then washed with double-distilled water to increase the density of OH-groups on the surface. The sample plates were then immersed in a mixture of water–acetonitrile (2 : 3 v/v), and a few drops of trimethylamine solution in acetonitrile (10% v/v) was added in order to catalyze the proton transfer in the course of the reaction. 0.5 M solution of cyanogen bromide in 10 ml of acetonitrile was added dropwise for 1 min. Then 1.5 ml of 10% (v/v) triethylamine solution in acetonitrile was added. The reaction mixture was allowed to stand for 20–30 min. To immobilize lysozyme, the modified plates were washed in double-distilled water and then immersed into the lysozyme solution (0.15 mg ml^{-1}) in 0.1 M carbonate buffer (pH 8.8), containing 0.5 M sodium chloride, for 3 h at room temperature. To remove the unreacted protein, the plates were washed in carbonate buffer for 10 h. The free CN-ester residues were blocked with 0.2 M glycine–sodium hydroxide solution (pH 8.0) for 2 h at room temperature. Afterwards, the sample plates were washed several times with 2 M sodium chloride solution and with plenty of double-distilled water.

The reaction of the immobilized protein with freshly prepared *trans*-4,4′-bis-brommethylstilbene was proceeded

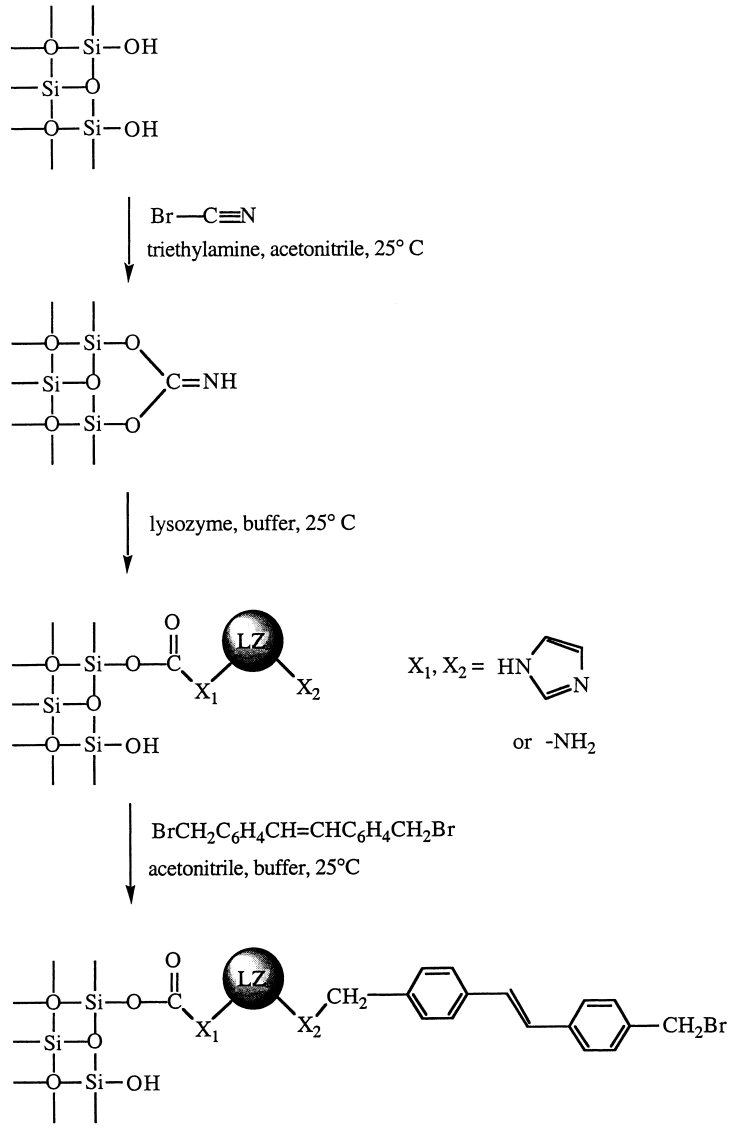

Fig. 3. Cyanogen bromide activation of quartz/silica surface and following it coating with lysozyme protein for stilbene immobilization to the surface.

at 35°C for 4 h. In detail, the sample plates, modified with lysozyme previously, were placed in 0.1 M carbonate buffer solution (pH 8.8). The saturated solution of *trans*-4,4′-bis-brommethylstilbene in acetonitrile was added. Attention must be paid to the fact that the stilbene solution in acetonitrile has to be concentrated as much as possible in order to reduce amount of organic solvent in the mixture. Finally, the plates were washed in water–acetonitrile mixture (10 : 1 v/v) to remove the unreacted stilbene and stored at 5°C in phosphate buffer solution (pH 7.2). Fig. 3 shows this procedure schematically .

2.2.3. Fluorescence measurements

Fluorescence excitation and emission spectra of the modified quartz plates were recorded with an SLM AB2 Amino-Bowman spectrofluorimeter. Each plate was placed into a quartz cuvette (1cm × 1 cm) with a specific holder at the excitation light angle of 45° taking into account that the fluorescence emission angle is 90°. The cuvette was filled with a solvent to cover the plate. The samples were degassed with argon before the measurements. All the measurements were performed at room temperature.

Fluorescence emission of *trans*-4-dimethylamino-4′-aminostilbene from each plate was recorded at $\lambda_{em} = 430$ nm after excitation near its absorption maximum at $\lambda_{ex} = 370$ nm (toluene) using typically a 16 nm slit width for both excitation and emission at constant-illumination conditions. The average spectrum from 12 sample plates was calculated. *Trans*-4,4′-bis-brommethylstilbene was excited at $\lambda_{ex} = 335$ nm (water) near its absorption maximum which was almost not at all dependent on the solvent. Constant-illumination fluorescence decay spectrum of this stilbene was recorded at $\lambda_{em} = 390$ nm. It was also run from 12 sample plates and the average spectrum was also calculated.

3. Results and discussion

The specific surface of plain quartz-contaminated plates was modified with two stilbene derivatives, which are fluorescent-photochrome labels, as was described above.

A commonly accepted model for *trans–cis* isomerization of stilbene assumes a one-dimensional reaction coordinate θ, which is the torsion (twist) angle about the olefinic double bond [11,12,14]. The transition state allowed for the *trans–cis* isomerization in the excited singlet state is expected to be polarizable and involve zwitterionic structures which lower the barrier to the torsional motion facilitating the isomerization reaction. That is why the *trans–cis* isomerization rate is dependent on the solvent polarity and viscosity. The apparent rate constant of *trans–cis* photoisomerization can be expressed in general case as follows:

$$k_{app} = \frac{\sigma I_o \phi_o k_{iso} k_{nr}}{(k_r + k_{iso})(k_r + k_{nr})} \quad (1)$$

where σ is the absorption cross-section, I_o the intensity of the incident light, ϕ_o the quantum yield of excited state, k_{iso} the isomerization rate constant, k_r and k_{nr} the radiative and non-radiative decay rate constants, respectively.

Previously, we have shown [26] than if the non-radiative medium relaxation process is substantially slower than the radiative deactivation of the excited molecule, i.e., $k_{nr} \ll k_r$, and the isomerization proceeds faster than the excited state deactivation, i.e., $k_{iso} \gg k_r$, then the apparent rate constant of *trans–cis* photoisomerization may be expressed as

$$k_{app} = \frac{\sigma I_o \phi_o k_{nr}}{k_r} \quad (2)$$

Otherwise, when the radiative deactivation of the excited state proceeds much more faster than the photoisomerization, $k_r \gg k_{iso}$, and much more slower than the medium relaxation, $k_r \ll k_{nr}$ then

$$k_{app} = \frac{\sigma I_o \phi_o k_{iso}}{k_r} \quad (3)$$

Eq. (2) corresponds to the medium of high viscosity where the process of relaxation is the rate-limiting state and its rate-constant value k_{nr} is close to the apparent rate constant k_{app}. Therefore, it is possible to study the dynamics of the medium in the vicinity of a stilbene label by measuring k_{app}, if the values of σ, I_o and k_r are known or k_{app} can be calibrated in a medium of known microviscosity.

In a low-viscous medium, the 'proper' isomerization is the rate-limiting step, and k_{app} which comes closer to the k_{iso} value, can be calculated with Eq. (3). In this case, k_{app} is dependent mainly on the chemical structure of the stilbene label, its donor–acceptor properties and micropolarity of the surroundings.

We have failed to detect the stilbene fluorescence from the plates surface after the direct silanization and following cross-linking of *trans*-4-dimethylamino-4′-aminostilbene through cyanuric chloride (Fig. 1). Immobilization of this stilbene label cross-linked with cyanuric chloride to the surface coated with bovine serum albumin (Fig. 2) gave us better results. Fig. 4 presents the fluorescence emission spectrum obtained in the case. It markedly differs from that in solution. The maximum fluorescence wavelength of the stilbene label in solution $\lambda_{em} = 398$ nm (toluene) is red-shifted to $\lambda_{em} = 417$ nm (toluene) for the immobilized stilbene measured at the same illumination conditions. This shift to the red is obvious because the protein–water environment accumulated on the quartz is much more polar than the toluene–bulk surroundings. The spectrum of the immobilized form becomes essentially broader compared to the free stilbene in solution because of the non-homogeneous distribution of the stilbene molecules at the protein and quartz surface. On the contrary, the fluorescence maximum of the immobilized stilbene label measured in glycerin $\lambda_{em} = 415$ nm (Fig. 4) is blue-shifted compared to the previously measured value of the same stilbene dissolved in glycerin $\lambda_{em} = 427$ nm as it was expected [26].

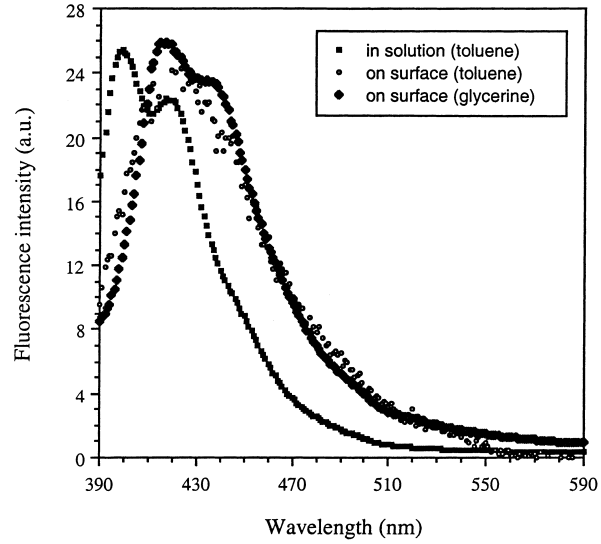

Fig. 4. Fluorescence emission of *trans*-4-dimethylamino-4′-amino-stilbene in a free state in 8×10^{-9} M toluene solution (closed squares) and an immobilized state cross-linked with cyanuric chloride to the coating BSA protein in toluene (open squares) and in glycerine (rhombus).

Previously, it has been shown that the maximum fluorescence wavelength of the stilbene chromophore was linearly dependent on the appropriate solvent polarity [11,12]. The dependence of the fluorescence emission energy E_f on the empirical solvent polarity parameter E_T^{30} introduced by Reichardt [36] is shown in Fig. 5. In contrast, the fluorescence maximum of the immobilized stilbene label measured in different solvents (Table 1) is not markedly sensitive to the solvent polarity (Fig. 5). These results support the idea that mainly silicaprotein surroundings exert influence on the relaxation processes of the excited stilbene molecules. This means that the stilbene molecules are buried in a protein–silica environment and the photochemical behavior of the immobilized stilbene molecules is not sensitive to the

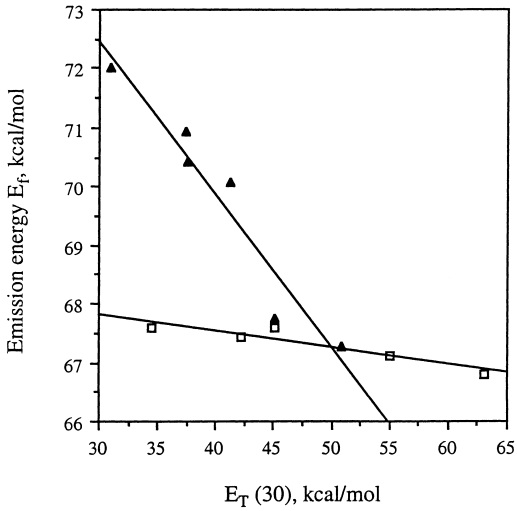

Fig. 5. Dependence of the fluorescence emission energy of *trans*-4-dimethylamino-4′-aminostilbene on the empirical solvent polarity parameter E_T^{30} [36] in the immobilized state (squares) and freely in solution (triangles) [26].

polarity of the bulk solvent. The experimental data presented in Fig. 5 allow to estimate the apparent local micropolarity in the vicinity of the stilbene label which is close to $E_T^{30} = 50$ kcal mol^{-1}.

A detailed investigation of the microviscosity effect on the photoisomerization behavior of the immobilized stilbene was carried out with the quartz plates modified with *trans*-4,4′-bis-brommethylstilbene according to the procedure outlined above (Fig. 3). The experimental fluorescence decay of *trans*-4,4′-bis bromethylstilbene irradiated at constant-illumination conditions in the mixture of glycerin–water with different glycerin/water compositions is shown in Fig. 6.

The apparent photoisomerization rate constant, k_{app}, is directly calculated from the constant-illumination fluorescence decay kinetics, as a slope of the first-order kinetic equation plotting dI_{fl}/dt against I_{fl}, where I_{fl} is the momentary intensity of the *trans*-isomer fluorescence and t the time of light exposure at the absorption maximum.

$$\frac{dI_{fl}}{dt} = -k_{app} I_{fl} + \text{constant} \quad (4)$$

The experimental rate constants of the fluorescence decay for *trans*-4,4′-bis-brommethylstilbene irradiated in a free state in solution and in its immobilized form are collected in Table 2. It should be mentioned that the photoisomerization constant for the free label is about three times higher than that for the immobilized molecule, because the protein and the quartz surface cause steric hindrances to rotation of the excited stilbene fragment around the olefinic bond. That is why the rate of photoisomerization is reduced for the immobilized stilbene label. The experimental value of the photoisomerization rate constant was found to be logarithmically dependent upon the medium viscosity. The logarithmic dependence of k_{app} on the reciprocal absolute viscosity $1/\eta$ of the bulk mixture glycerin–water is shown in Fig. 7. We guess that the pre-logarithmic factor, which is two times less for the immobilized molecule also indicates the hindrances for rotation around the olefinic bond in the excited state. The rotation of aromatic moieties is restricted by the protein or plate surface which markedly reduce the photoisomerization rate. The curve plotted in Fig. 7 may be used as a calibration curve for the microviscosity determination in the vicinity of the fluorescence-photochrome probe. This technique is very sensitive compared to other well-known techniques for the determination of microviscosity, like spin- or triplet-photochrome methods. It allows to measure the microviscosity in the vicinity of the immobilized chromophore with the minimal estimated volume concentration of about 10^{-9}–10^{-8} M, which corresponds approximately to 3×110^{11}–3×10^{12} fluorescent molecules per surface of each modified quartz plate.

Table 1
Solvent polarity E_T^{30} (kcal/mol), fluorescence wavelength λ_{max} (nm) and emission energy E_f (kcal/mol) of *trans*-4-dimethylamino-4′-aminostilbene measured in solution and in an immobilized state on a quartz-contaminated surface coated with bovine serum albumin

Solvent	Fluorescence wavelength, λ_{max} (nm)	Emission energy, E_f (kcal/mol)	E_T^{30} (kcal/mol)
Solution measurements			
Cyclohexane	397	72.01	30.9
THF	403	70.94	37.4
Chlorobenzene	406	70.42	37.5
MEK	408	70.07	41.3
DMSO	422	67.75	45.1
Benzyl alcohol	425	67.27	50.8
Surface measurements			
Toluene	423	67.59	34.5
Acetone	424	67.43	42.2
DMSO	423	67.59	45.1
Methanol	426	67.11	55.5
Water	428	66.80	63.1

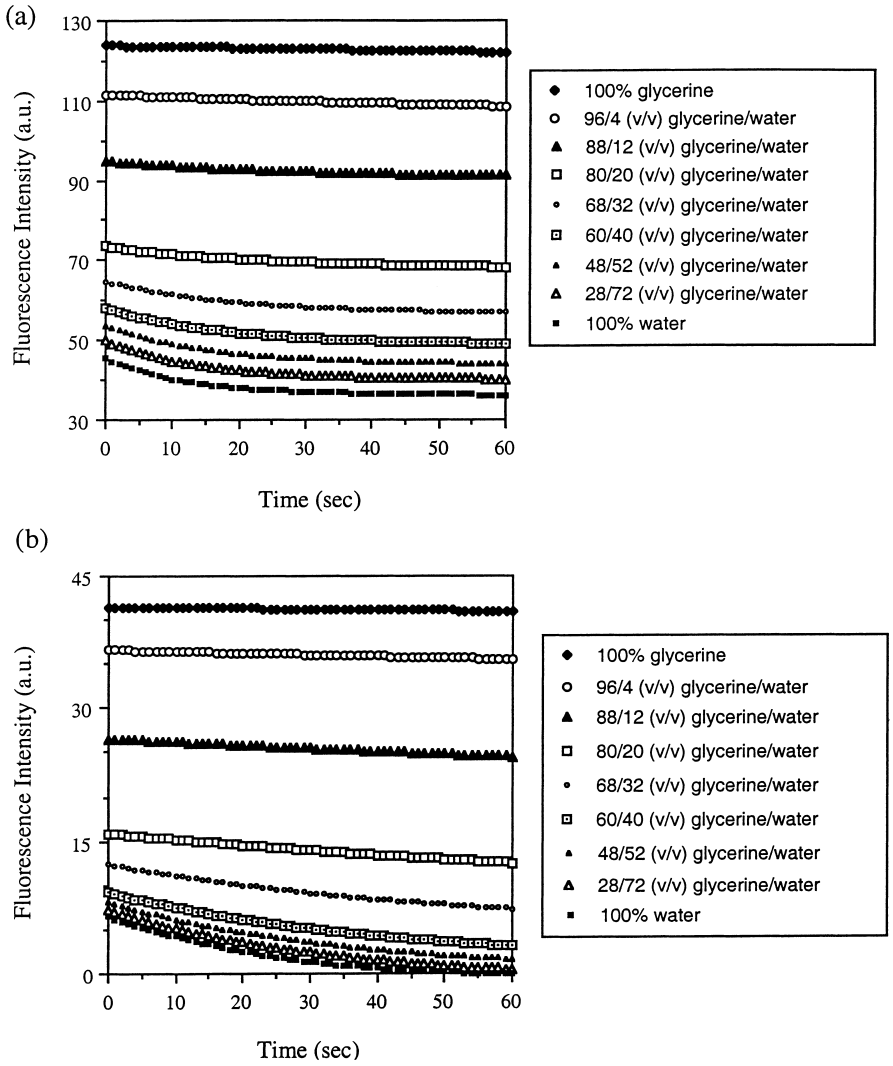

Fig. 6. Fluorescence decay kinetics of *trans*-4,4′-bis-brommethyl-stilbene at constant-illumination conditions in the mixture of glycerine–water with different glycerine/water (v/v) compositions (a) for a free stilbene label in solution (2×10^{-6} M), (b) for the immobilized stilbene derivative on the silica/quartz surface.

Table 2
Composition of a water–glycerine mixture (% v/v), its density, absolute viscosity and experimental rate constants of the fluorescence decay for *trans*-4,4′-bis-brommethyl-stilbene

Water–glycerine composition (%)	Absolute viscosity, η (cP)	$k_{app} \times 10^3$ (solution) ($M^{-1}\ s^{-1}$)	$k_{app} \times 10^3$ (surface) ($M^{-1}\ s^{-1}$)
100/0	1.000	78.0	34.1
72/28	2.003	75.0	32.2
52/48	4.315	65.4	28.3
40/60	8.035	58.1	23.9
32/68	13.36	49.6	18.9
20/80	41.02	41.5	9.21
12/88	97.49	31.8	4.94
4/96	489.9	15.7	2.55
0/100	1179	4.51	1.05

The analysis was done in both cases: (1) being dissolved in a water–glycerine solution, (2) being immobilized at the silica/quartz surface through protein conjugate.

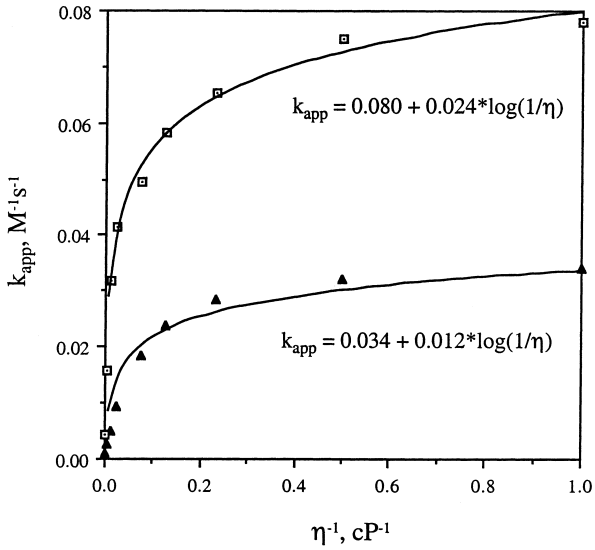

Fig. 7. Logarithmic dependence of the *trans–cis* photoisomerization rate constant k_{app} on the reciprocal absolute viscosity $1/\eta$ of the water–glycerol mixture for the stilbene derivative in solution (squares) and for the immobilized stilbene label (triangles).

4. Conclusions

In the present work, the fluorescence-photochrome technique was applied to investigate the solid surface which is quartz-contaminated and modified with the fluorescence emission of the stilbene derivative and its *trans–cis* photoisomerization responsible for the fluorescence decay. While the direct silanization was not successful, the immobilization of stilbene labels cross-linked with cyanuric chloride to the surface coated with proteins allowed us to measure the fluorescence from the plate surface and follow the kinetics of *trans–cis* photoisomerization.

The photochemical behavior of the immobilized stilbene label is markedly differed from that in solution. Fluorescence spectrum of the immobilized label measured in toluene was red-shifted because of the protein–water environment accumulated on the quartz surface which is much more polar than the toluene–bulk surroundings. Studies of the solvent polar effects on the excited state of the immobilized stilbenes indicate that the maximum wavelength of the fluorescence emission is not sensitive to the solvent polarity. The fluorescence emission energy of the free label in solution E_f exhibits the linear dependence on the empirically determined E_T^{30} solvent parameter. The apparent local polarity of the medium in the vicinity of the stilbene label was estimated and the 'virtual' solvent polarity parameter E_T^{30} was found to be approximately 50 kcals mol^{-1}.

The photoisomerization kinetics of the stilbene label in its immobilized and free states in a medium of different viscosity was monitored with the fluorescence technique at the constant-illumination conditions. The apparent photoisomerization rate constant of the process was calculated using immobilized label than in a free state which indicates that the surface and protein itself sterically hinder the rotation of the stilbene fragment around the olefinic double bond in the excited state. Investigation of the microviscosity effect on the photoisomerization of the immobilized and free stilbene label was carried out changing the relative concentration (% v/v) of glycerin–water mixture used as a solvent. The apparent *trans–cis* photoisomerization rate constant was found to be logarithmically dependent on the reciprocal value of absolute viscosity of the glycerol–water solution for both the immobilized and free molecule.

The viscosity dependence of this behavior suggests that stilbene molecules can be useful indicators of the local microviscosity in various condensed media. The logarithmic dependence of k_{app} on the reciprocal absolute viscosity $1/\eta$ of the solvent may be used as a calibration curve for the microviscosity defemination in the vicinity of the fluorescence-photochrome probe.

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